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Research paper

Single-chain Fv antibody with specificity for *Listeria monocytogenes*☆

George C. Paoli[a], Chin-Yi Chen[b], Jeffrey D. Brewster[a,*]

[a] *Microbial Biophysics and Residue Chemistry and Core Technologies, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Service, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA*

[b] *Microbial Food Safety Research Units, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Service, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA*

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Abstract

Single chain antibodies (scFv) exhibiting specific binding to *Listeria monocytogenes* strains were isolated from a pool of random scFvs expressed on the surface of filamentous bacteriophages. Positive selection (panning) using *L. monocytogenes* was used to enrich for phage clones with the desired binding affinity, and negative selection using *L. innocua* and *L. ivanovii* was used to remove phages expressing cross-reactive antibody fragments. A single phage clone, P4:A8, was selected using two independent panning schemes. A rapid assay was devised to determine phage antibody binding specificity and was used to develop a selectivity profile for individual phage clones. The P4:A8 clone was screened against a panel of bacteria consisting of eight strains of *L. monocytogenes*, one each of the other six species of *Listeria* and nine other relevant bacterial species. A collection of individual clones from the penultimate panning was also screened against a subset of the panel of bacteria. The selectivity profiles indicate that multiple clones, including P4:A8, exhibit binding to one or more strains of *L. monocytogenes* without cross-reactivity toward any other species in the panel. This is the first report of a species-specific antibody for viable cells of *L. monocytogenes* (i.e., the ability to bind to *L. monocytogenes* without cross-reactivity toward any other species of *Listeria*).
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Keywords: Phage display; *Listeria monocytogenes*; Pathogen detection; scFv

1. Introduction

Modern food production and distribution systems permit nationwide distribution of large quantities of food in a few days. These systems provide consumers with consistent, low-cost, high quality foods, but may also be a conduit for exposing large populations to pathogens introduced inadvertently, or by deliberate

Abbreviations: scFv, single chain antibody fragment; PBS, phosphate-buffered saline; TTBS, Tris-buffered saline containing 0.01% Tween 20; BSA, bovine serum albumin; tu, transforming units.

☆ Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

* Corresponding author. Tel.: +1-215-233-6447; fax: +1-215-233-6559.
E-mail address: jbrewster@errc.ars.usda.gov (J.D. Brewster).

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acts of bioterrorism, into foodstuffs. There is intense interest in rapid assays which can detect harmful levels of pathogens in hours rather than days as required by conventional culture methods. *Listeria monocytogenes*, the only species of *Listeria* which is pathogenic in humans, is of particular interest because it is able to survive and grow at low temperatures and because the mortality rate for infected individuals is much higher than for other common food-borne pathogens.

Virtually all rapid assay methods make use of the high affinity and selectivity of antibody–antigen interactions. Antibodies are frequently used for isolation and concentration of pathogens from the sample matrix prior to detection. Immunomagnetic beads (Skjerve and Olsvik, 1991; Luk and Lindberg, 1991; Fratamico et al., 1992; Gehring et al., 1996) are very widely used, and related approaches such as immunoaffinity columns (Brewster, 2003), and immunofiltration membranes (Paffard et al., 1996; Gehring et al., 1998) have also been developed. The purified, concentrated bacteria may be detected immediately, or cultured to further increase the bacterial concentration, establish viability, or provide confirmation by conventional methods. Antibody used for this purpose must bind to the surface of intact, viable cells. Many methods utilize labeled antibodies as signal generation reagents (Feng, 1996), exploiting such diverse detection modes as absorbance (Wyatt et al., 1993), epifluorescence microscopy (Tortorello and Gendel, 1993), fluorescence (Tu et al., 2001), amperometry (Hadas et al., 1992; Brewster and Mazenko, 1998), and electrochemiluminescence (Yu and Bruno, 1996; Crawford et al., 2000). Antibody for detection purposes may be directed against surface as well as internal antigens.

Although researchers have developed a wide variety of innovative and effective approaches for rapid pathogen detection, their application has been restricted to the few target bacteria for which appropriate antibodies are available (*E. coli* O157:H7 and *Salmonella* spp.). Development of rapid assays for important pathogens such as *L. monocytogenes* and *Campylobacter* has been thwarted by the lack of specific, high affinity antibody. The importance of *L. monocytogenes* as a food-borne human pathogen has led to extensive efforts to develop a species-selective antibody (Nannapaneni et al., 1998a,b; Mattingly et al., 1988; Curiale et al., 1994; Bubert et al., 1994; Bhunia, 1997). Hundreds of publications over the past two decades have focused on this task, and numerous approaches have been pursued to produce a polyclonal or monoclonal antibody with the appropriate selectivity (Bhunia, 1997). Success has been elusive, suggesting that *L. monocytogenes* either lacks surface epitopes which are both unique and antigenic, or that such epitopes are not effectively processed or presented during in-vivo antibody production and maturation. Antibody phage display techniques (Winter et al., 1994) do not rely on antigen processing and presentation, and may provide a route to success where conventional techniques have failed.

Phage display has proven a useful tool for the isolation of antibody fragments with desired specificities. The technique involves the display of a library of single-chain antibody (scFv) fragments on the surface of filamentous phages followed by selection of the desired recombinant phages by means of specific binding to an antigen of interest. Although phage display has advantages over conventional polyclonal and monoclonal antibody production, few reports (Benhar et al., 2001) have employed this technique for the selection of reagents for the detection of food-borne pathogens.

Phage display also provides several approaches for rational improvement of antibody affinity and selectivity. Given a set of phage clones with known affinity and selectivity profiles, selection strategies can be designed to isolate clones with optimal properties from the existing library of clones. By correlating affinity and selectivity data with DNA sequence information, it is feasible to design and construct novel sequences that express antibodies with the desired properties.

We present here the results of the first stage in this process of generating a species-specific immunological reagent for the detection of *L. monocytogenes* in food. Using live *L. monocytogenes* cells to select phage antibodies and cells of other *Listeria* spp. to remove cross-reacting phages, a single phage antibody clone was isolated. This phage antibody bound some strains of *L. monocytogenes* without cross-reactivity toward any other species of *Listeria*. This is the first report of a species–specific antibody for viable cells of *L. monocytogenes*.

2. Materials and methods

2.1. Materials

Water was deionized in-house with a Nanopure water treatment system (Barnstead, Dubuque, IA). The Griffin.1 library (Human Synthetic VH+VL scFv Library) and the phage host strain *Escherichia coli* TG1 were the gift of the Centre for Protein Engineering (Cambridge, UK). Bacteriological media and agar were from Difco (Detroit, MI, USA). All other chemicals used were of reagent grade.

2.2. Bacterial cultures and media

E. coli TG1 was used to propagate phages. *E. coli*, *Salmonella typhimurium*, and *Pseudomonas putida* were grown in Luria Broth (LB, Difco) or LB containing 1% glucose at 37 °C. During phage propagation, ampicillin and kanamycin were used as necessary at a final concentration of 100 and 25 μg/ml, respectively. *Listeria* spp. were grown in Brain-Heart-Infusion (BHI, Difco) at 37 °C. All aerobic cultures were grown with shaking at 250 rpm. *Lactobacillus* spp. and *Pediococcus acidilactici* were grown in MRS Medium (Difco) in stationary tubes at 37 and 30 °C, respectively. *Clostriudium perfringens* was grown in BHI in stationary tubes at 37 °C. *Streptococcus thermophilus* was grown in TYL broth in stationary tubes at 37 °C. *Campylobacter jejuni* was grown at 42 °C on Meuller-Hinton Base (Difco) agar plates in BBL GasPak Jars (Becton, Dickinson and Company, Franklin Lakes, NJ USA) using the CampPak system (Becton, Dickinson and Company).

To provide a uniform stock of cells for suspension affinity assays, washed cell suspensions were prepared and stored as follows. A starter culture was prepared by inoculating 3 ml of BHI with a single colony of the desired bacteria and grown overnight at 37 °C with shaking at 250 rpm. A 200 ml flask containing 50 ml of BHI was inoculated with 0.5 ml of the overnight starter culture and grown at 37 °C with shaking at 250 rpm. After incubating for 4 h, the log phase cells were harvested by centrifugation at 3000 rpm (Sorvall RT6000B with Sorvall A500 rotor, Kendro Laboratory Products, Newtown, CT, USA) for 10 min. The supernate was discarded, the cell pellet was resuspended in 50 ml Tris-buffered saline containing 0.01% Tween 20 (TTBS) and cells were again collected by centrifugation. After discarding the supernate, the cell pellet was resuspended in TTBS containing 20% glycerol to an absorbance at 600 nm of 1 and stored at −10 °C. Prior to use in the affinity assay, glycerol was removed from a 1 ml aliquot of the cell stock by spinning in a microcentrifuge at 13,000 rpm for 2 min, discarding the supernate, and resuspending in 1 ml TTBS.

2.3. Apparatus

An EL 311e Microplate reader (Bio-Tek Instruments, Winooski, VT, USA) equipped with a shaker and temperature control unit was interfaced to a laptop computer and used to read the absorbance (A) of microwell plate samples. A Beckman Coulter Avanti J-25 centrifuge (Beckman Coulter, Fullerton, CA USA) with a JS16.25 rotor was used for harvesting large volumes of cells and a JA25.50 rotor was used for phage preparation. A Heraeus Biofuge microcentrifuge (Kendro Laboratory Products) was used for centrifugation of small volumes.

2.4. Panning

The panning protocol is outlined in Fig. 1. For the positive pannings, an overnight culture of *L. monocytogenes* ATCC 19115 was diluted 1/50 in 5 ml of BHI and incubated with shaking until it reached an A at 600 nm of approximately 1 (4–5 h). One milliliter (10^9 cells) of the culture was washed twice with phosphate-buffered saline (PBS), resuspended in 1 ml of PBS containing 1% BSA, and incubated in a 1.5 ml microcentrifuge tube on a rotator for 1 h at room temperature. This and subsequent incubations were carried out using microcentrifuge tubes that had been blocked with 1% BSA in PBS for 1 h at room temperature. After 1 h, 10^{12} phage particles were added to the tube containing 10^9 cells in PBS-BSA and rotated for an additional 1 h at room temperature to allow the phages to bind to the cells. Unbound phages were removed by washing the cells five times with PBS containing 0.05% Tween-20 followed by five washes with PBS. For the final wash step the resuspended cells were moved to a fresh tube that was blocked with 1% BSA in PBS. This step

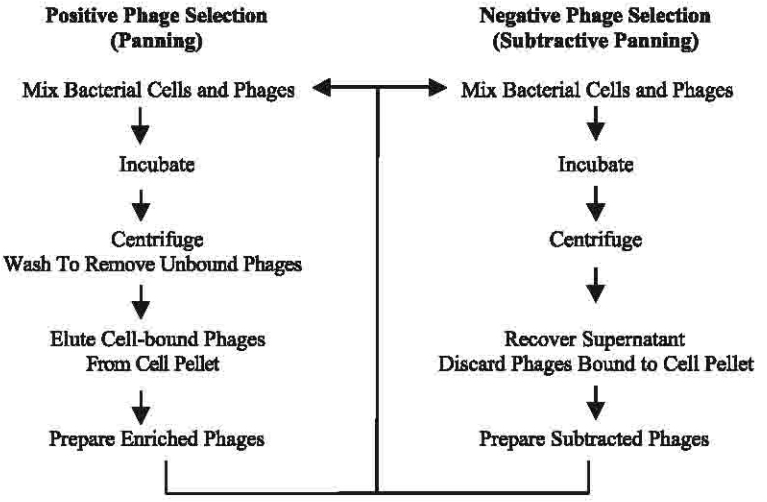

Fig. 1. Positive and negative phage panning using whole bacterial cells.

eliminated the possibility of eluting phages that had bound to the microcentrifuge tube. After the final spin, the cell pellet was resuspended in 500 μl of 50 mM citrate buffer (pH 2.6) and rotated at room temperature for 5 min, to elute the bound phages. The tube was spun for 2 min at 13,000 rpm and the supernatant containing the eluted phages was transferred to a new microcentrifuge tube and neutralized with 200 μl 1M Tris–HCl pH 8.0. The neutralized eluted phage solution was stored on ice.

L. ivanovii ATCC 49954 and *L. innocua* ATCC 51742 were used for subtractive pannings. Cells (10^9 of both strains) were pooled, and washed and blocked as described above. After the cells were rotated for 1 h in at room temperature, 10^9 phage particles were added to the microcentrifuge tube and the mixture was rotated for 1 h at room temperature. The tube was spun for 2 min at 13,000 rpm in a microcentrifuge tube. The supernatant, containing the unbound phages, was transferred to a new tube and stored on ice. The cell pellet, containing the subtracted phages, was discarded.

Phage propagation and titering were conducted as described by Griffiths et al. (1994).

2.5. Immobilized-bacteria binding specificity assay

The extent and specificity of phage binding to *L. monocytogenes* was estimated with a sandwich ELISA using passively adsorbed bacteria as the capture phase and anti-phage antibody for detection of bound phages. An overnight culture of *L. monocytogenes* ATCC 19115 was diluted 1/100 into fresh BHI and grown to an A_{600} of 1 (4–6 h). The cells were washed twice with PBS and resuspended in PBS to 10^8 cells/ml (the A_{600} of 10^9 cells/ml was 1). Polystyrene microtiter plates were coated with 150 μl of the washed bacterial cell suspension for 1 h at 37 °C followed by an overnight incubation at 4 °C. The next day, the plates were washed three times with 200 μl of PBS and blocked with 200 μl of 2% MPBS (PBS containing 2% powdered milk) for 1–3 h. After washing three times with 200 μl PBS, 150 μl of the phage preparation (10^9 tu/ml in 2% MPBS) were added and the plate was incubated at room temperature for 1 to 2 h. The phage solution diluted into 2% MPBS was stored at 4 °C for 15–30 min prior to use in order to block the phage proteins and prevent non-specific binding of the phages to the cells in the plates. After incubation with the phage solution the plates were washed three times with 0.01% PBST (PBS containing 0.01% Tween 20) followed by an additional three washes with PBS. The cell bound phages were detected using anti-M13 horseradish peroxidase-conjugate (Amersham Biosciences, Piscataway, NJ, USA). The commercial antibody preparation was diluted 1/5000 in 2% MPBS and 150 μl of the diluted antibody was added to each well. The plates were incubated at room temperature

for 1 h. Unbound antibody was removed by washing three times with 0.01% PBST (0.01% Tween 20 in PBS) followed by an additional three washes with PBS. The horseradish peroxidase (HRP) activity was determined using ABTS-substrate as described by the manufacturer (Amersham Biosciences). The color change at 405 nm was monitored every 5 min for 2 h using an EL 311e Microplate reader (Bio-Tek Instruments).

2.6. Suspended-bacteria binding specificity assay

The extent and specificity of phage binding to the bacteria in Table 1 was estimated using a novel assay in which a bacteria–phage–antibody complex was formed in the liquid phase and separated by centrifugation. Prior to use, bacteria from a stock suspension were washed by spinning in a microcentrifuge at 13,000 rpm for 2 min, discarding the supernate, and resuspending in TTBS to A_{600} of 1. Washed bacteria (100 μl), phages (10^{10} tu), and anti-M13-HRP conjugated antibodies (100 μl of 1/5000 dilution) were placed in a 1.5 ml microcentrifuge tube and brought to a volume of 500 μl with TTBS. After incubation at 4 °C for 2 h on a rotating mixer, the cells (with bound phages and HRP conjugated antibodies) were collected by centrifugation at 13,000 rpm for 2 min. The supernate was transferred to a fresh tube and the cells washed twice by resuspension in 250 μl TTBS, centrifugation, and removal of the supernate. The pellet was resuspended in 250 μl TTBS. Samples of the washed suspension (4 × 25 μl) were combined with 150 μl TMB substrate (Amersham Biosciences) in a microwell plate and the absorbance measured at 630 nm with the plate reader. Enzyme activity was determined from the change in absorbance over time. In some experiments samples of the combined supernatants and washes (4 × 25 μl) were also assayed to assess reproducibility.

Table 1
Selectivity of phage clone P4:A8 as determined by ELISA

Bacterial species	Strain	Source[a]	ELISA response
Listeria monocytogenes	ATCC 19115	ATCC	+
Listeria monocytogenes	F2365	MFS	+
Listeria monocytogenes	N3-008	WRRC	–
Listeria monocytogenes	Lm4085	MFS	+
Listeria monocytogenes	ATCC 19113	PUDFS	–
Listeria monocytogenes	ATCC 19114	PUDFS	+
Listeria monocytogenes	ATCC 19116	PUDFS	+
Listeria monocytogenes	Scott A	MFS	+
Listeria grayi	ATCC 700545	ATCC	–
Listeria innocua	ATCC 51742	MFS	–
Listeria ivanovii	ATCC 89954	ATCC	–
Listeria murrayi	F4076	MFS	–
Listeria seeligerii	F4880	MFS	–
Listeria welshimeri	CF1LP	MFS	–
Streptococcus thermophilus	ATCC 19258	DPP	–
Escherichia coli O157:H7	B1409	MBRC	–
Salmonella typhimurium	14028	MBRC	–
Lactobacillus plantarum	ATCC 14917	DPP	–
Lactobacillus bulgaris	ATCC 11842	DPP	–
Pediococcus acidilactici	F	DPP	–
Campylobacter jejuni	81-176	MFS	–
Pseudomonas putida	KT-2442	FOAC	–
Clostridium perfringens	H6	MFS	–

[a] ATCC—American Type Culture Collection; DPP—Dairy Processing and Products Research Unit of the United States Department of Agriculture (USDA) Eastern Regional Research Center (ERRC); FOAC—Fats, Oils and Animal Coproducts Research Unit of the USDA ERRC; MFS—Microbial Food Safety Research Unit of the USDA ERRC; MBRC—Microbial Biophysics and Residue Chemistry Research Unit of the USDA ERRC PUDFS—Purdue University Department of Food Science; WRRC—USDA Western Regional Research Center.

3. Results

3.1. Pannings

Phage antibody selections were conducted as described above using the scheme shown in Fig. 2. An increase in phage affinity was observed with sequential rounds of panning against whole cells of *L. monocytogenes* with a dramatic increase after the fourth panning (Fig. 3A). When the phage pool from the third panning was panned against *L. ivanovii* and *L. innocua* to subtract phage displaying antibody fragments bound to cell surface antigens that were not unique to *L. monocytogenes*, a modest drop in affinity was observed (Fig. 3B). Subsequent rounds of panning using *L. monocytogenes* resulted in an increase in affinity for the subtracted phage pool that was comparable to that of the phage pool derived from the positive panning (Fig. 3A and B). Ninety-six individual phage clones from the each of the final pannings [panning 4 (P4) for the positive selection scheme and panning 7 (P7) for the subtractive panning scheme, Fig. 2] were screened for binding to

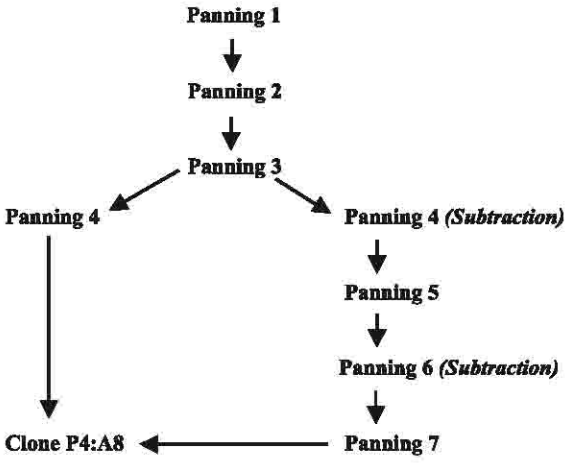

Fig. 2. Panning scheme. The phage antibody library was panned three times against *Listeria monocytogenes*. After the third panning, the enriched phage antibody preparation was panned once more against *L. monocytogenes* and subjected to the subtractive panning strategy using *L. ivanovii* and *L. innocua* as shown above. A single phage clone (P4:A8), isolated at the end of both panning shemes, was characterized.

immobilized *L. monocytogenes* ATCC 19115 (data not shown). The nucleotide sequence of the 6 P4 clones and 5 P7 clones showing the highest binding affinity was determined. Each of these clones was identical (accession number AY538748); therefore, only one clone (P4:A8) was characterized further.

3.2. Phage antibody reactivity profile

Table 1 illustrates the binding selectivity profile of clone A8 from the fourth panning (P4:A8) screened against a panel of *Listeria* strains and representative Gram-positive and Gram-negative bacteria from other genera. In the ELISA procedure, the observed signal reflected both the number of bacterial cells adsorbed to the microwell plate and the extent of phage-bacteria binding. To eliminate any effect of cell adsorption (which differed significantly among the tested organisms), the binding selectivity profiles were determined in the liquid phase using equal numbers of suspended cells. Clone P4:A8 exhibited binding to six of the eight *L. monocytogenes* strains and no cross-reactivity toward any of the other species tested.

The isolation of identical phage clones from pannings carried out with and without subtraction suggested that a single clone might have dominated the phage pool after the third panning (i.e., Lm:P3, the last phage pool common to both the positive and subtractive panning procedures). To investigate this possibility, 10 clones were selected at random and assayed for binding to representative *Listeria* spp. The results (Table 2) demonstrate that the Lm:P3 pool was very diverse, yet highly enriched in phage with affinity to *L. monocytogenes*. Seven of ten randomly picked clones detected at least one strain

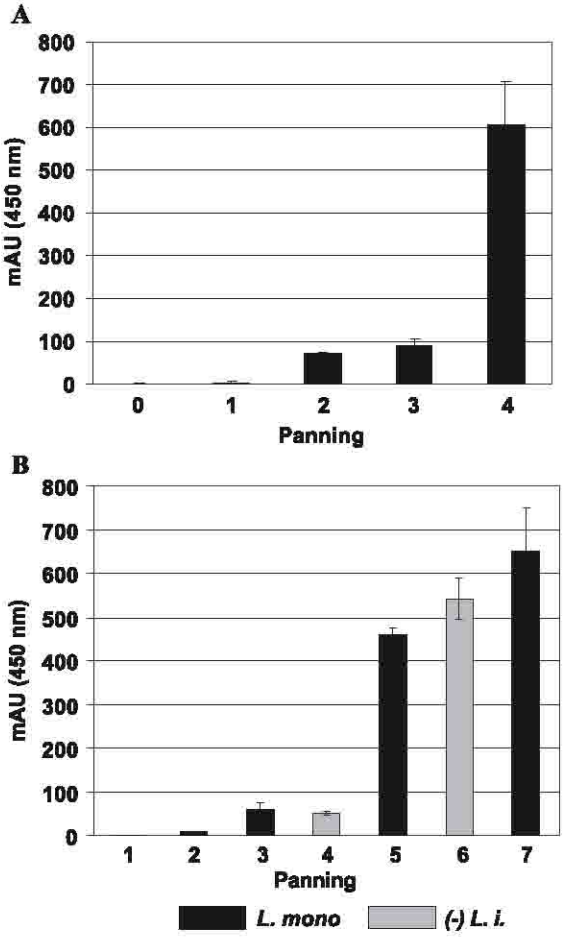

Fig. 3. Selection of Phage Antibodies Binding to *Listeria monocytogenes*. ELISA results for phage antibody from each round of panning. (A) Four rounds of panning against *L. monocytogenes*; (B) Seven rounds of panning with subtractive pannings as indicated in Fig. 2. Binding of phage antibodies to *L. monocytoegnes* coated microtiter plates was detected via HRP-conjugated anti-fd. Black bars indicate positive pannings against *L. monocytogenes* and gray bars indicate subtractvie pannings using *L. ivanovii* and *L. innocua*. The error bars indicate ± one standard deviation.

Table 2
Selectivity of Lm:P3 phage clones as determined by ELISA

Bacterial species	Strain	Clone P4:A8	Lm:P3 Clones									
			1	2	3	4	5	6	7	8	9	10
Listeria monocytogenes	ATCC 19115	+	–	+	+	–	–	–	+	–	+	–
Listeria monocytogenes	F2365	+	+	–	+	–	–	–	+	+	–	–
Listeria monocytogenes	N3-008	–	–	–	–	–	–	–	–	–	–	–
Listeria monocytogenes	Lm4085	+	+	+	+	–	+	–	+	+	+	–
Listeria innocua	ATCC 51742	–	–	–	–	–	–	–	–	–	–	–
Listeria ivanovii	ATCC 89954	–	–	–	–	–	–	–	–	–	–	–

of *L. monocytogenes*. Five different selectivity profiles were observed, ranging from no binding to any of the *Listeria* strains examined (clones 4, 6, and 10) to selectivities identical to P4:A8 (clones 3 and 7) (Table 2). Three of the Lm:P3 clones (clones 1, 5, and 8) yielded a positive response in the ELISA procedure with some strains of *L. monocytogenes*, but not with strain ATCC 19115 (Table 2), despite the fact that the latter strain was used for selection. This apparent discrepancy is most likely due to the differences in the moderate affinity required for selection versus the relatively high affinity required for detection via ELISA. A phage-antibody with high affinity for an epitope on strain F2365, and moderate affinity for a related epitope on the ATCC 19115 strain would result in selection and enrichment of this clone. However, an ELISA binding assay using the resulting clone would be negative for ATCC 19115 and positive for F2365. None of the clones exhibited detectable binding to *L. ivanovii* or *L. innocua*, indicating that the phage pool contained few, if any, cross-reactive clones. Thus, the isolation of identical phage clones after subsequent positive and negative panning schemes was not due to a lack of diversity in pool of phage derived from three rounds of enrichment.

4. Discussion

Conventional techniques have failed to produce antibodies with the specificity and avidity required for rapid, selective assays for the detection of *L. monocytogenes* and other important pathogens. Phage display provides promise of reaching this goal. The specific goals for this stage of our research were to develop suitable panning protocols and methodology for assaying binding affinity that would be applicable to a wide range of bacterial species and to apply these methods to select and evaluate species-specific phage displayed antibody for *L. monocytogenes*. The results reported here indicate that highly specific scFvs can be produced from a naïve phage display antibody library by panning against whole bacterial cells. The simple panning procedure took advantage of the fact that phage-antibodies bound to the bacterial cells can be removed from solution by centrifugation. Non-binding phages were discarded with the supernatant and phage-antibodies non-specifically bound to the cells were removed by washing the pellet containing cells and bound phages. Thus, it was not necessary to bind the cells to a solid surface or to have a purified antigen prior to panning. Difficulties which plague other panning methods, such as selection of phage binding to the support or the container were easily avoided, leading to efficient processing. One constraint of the suspended-cell panning procedure employed is that scFvs isolated by this procedure are limited to interactions with cell surface antigens. The panning strategy implemented here led to isolation of a single scFv with specific reactivity toward six *L. monocytogenes* strains and no detectable cross-reactivity toward other *Listeria* species or bacteria from other genera.

Another advantage of phage display technology is the ability to remove cross-reacting phage-antibodies via subtractive panning (Burioni et al., 1998; Shinohara et al., 2000; Stausbol-Gron et al., 1996). In the suspended-cell panning procedure described here, this was accomplished by incubating an enriched phage pool derived from 3 rounds of panning against *L. monocytogenes* with cells of other *Listeria* species, centrifuging the mixture, discarding the cell-phage pellet and saving the supernatant. *L. ivanovii* and *L.*

inoccua were selected for the subtraction because *L. ivanovii* is the only other pathogenic species of *Listeria* (Vazquez-Boland et al., 2001) and monoclonal antibodies raised against *L. monocytogenes* that cross-react with only *L. inoccua* have been described previously (Bhunia et al., 1991). Thus, subtraction with cells of these two species of *Listeria* would remove phages expressing cross-reacting scFvs. Surprisingly, subtractive panning with *L. ivanovii* and *L. innocua* yielded the same single *L. monocytogenes*-specific phage-antibody clone as was isolated from panning schemes without subtraction steps. Although unnecessary for this particular library/target combination, it is likely that the simple subtractive panning scheme described here will be needed to eliminate cross-reactive clones when developing scFv reagents for other target organisms.

As mentioned above, the suspended cell panning procedure is limited to the selection of antibody fragments directed against cell surface antigens. That limitation may have been a key advantage in the isolation of a *L. monocytogenes*-specific scFv. It is known that the humoral response to natural and experimental infections with *L. monocytogenes* is aimed primarily at extracellular proteins and a lack of antibodies to cell-surface antigens has been attributed to the intracellular nature of the *Listeria* infection (Bhunia, 1997; Edelson and Unanue, 2000). It is likely that the in vitro selection of *L. monocytogenes*-specific antibody fragment P4:A8 via phage display allowed the selection of this unique monoclonal phage-antibody fragment that binds to a cell surface protein to which infected hosts do not respond.

Based on the apparent ratio of bound/free phages from suspended cell experiments and the assumptions that 10% of the phages express a copy of the scFv and that the average cell carries 10 binding sites, we estimate the binding constant for *L. monocytogenes*-specific phage antibody P4:A8 binding to *L. monocytogenes* ATCC19115 as 10^8 M^{-1} (data not shown). Although this preliminary analysis indicates the affinity of this antibody is sufficient for applications in isolation and detection of *L. monocytogenes*, further improvement is desirable. The panning strategy yielded a pool of clones after the third panning which exhibit diverse specificity for *L. monocytogenes* strains and low cross reactivity toward other bacteria. This pool is expected to be an excellent source for the further refinement of specificity and avidity. Future work will utilize the specificity data to design and implement panning strategies aimed at producing a clone or clones capable of recognizing all strains of *L. monocytogenes* with full species specificity.

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